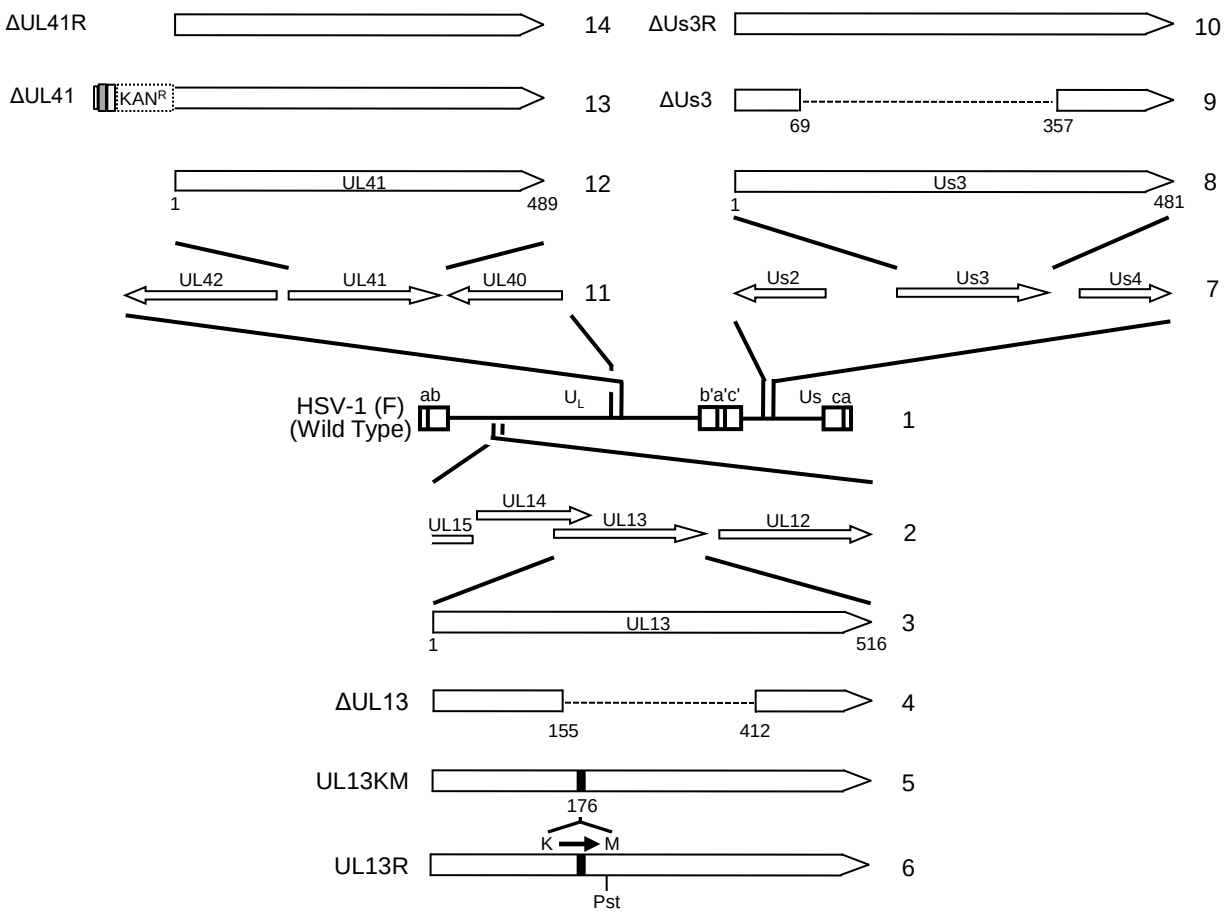
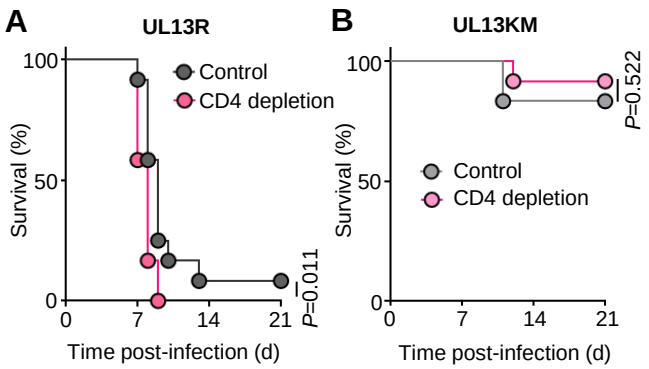
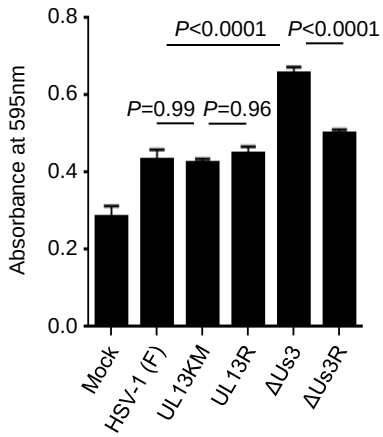


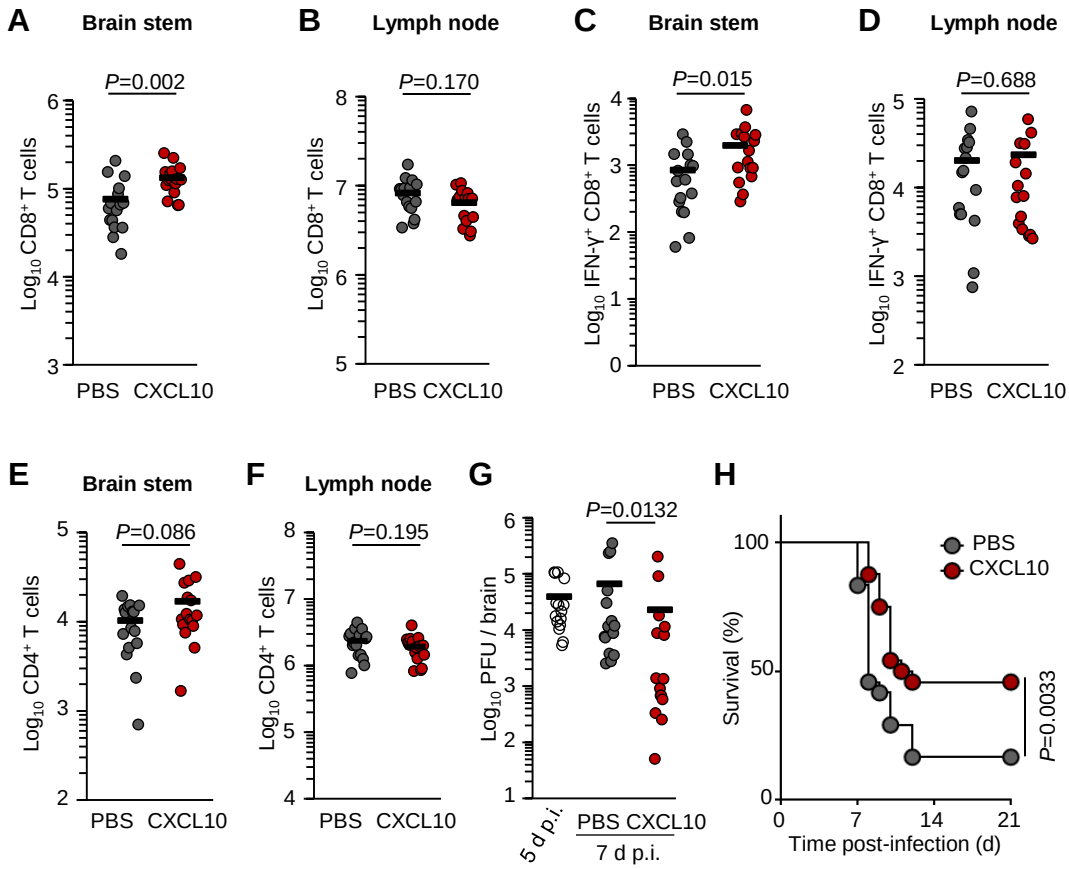
Supplementary Figure 1. Schematic diagrams of the genome structures of wild-type HSV-1(F) and recombinant viruses used in this study. Line 1, wild-type HSV-1(F) genome; line 2, domain encoding the UL12 to UL15 open reading frames (ORFs); line 3, domain of UL13; line 4 to 6, recombinant viruses with mutations in UL13; line 7, domain encoding Us2 to Us4 ORFs; line 8, domain of Us3; lines 9 and 10, recombinant viruses with mutations in Us3; line 11, domain encoding the UL40 to UL42 ORFs; line 12, domain of UL41; lines 13 and 14, recombinant viruses with mutations in UL41.



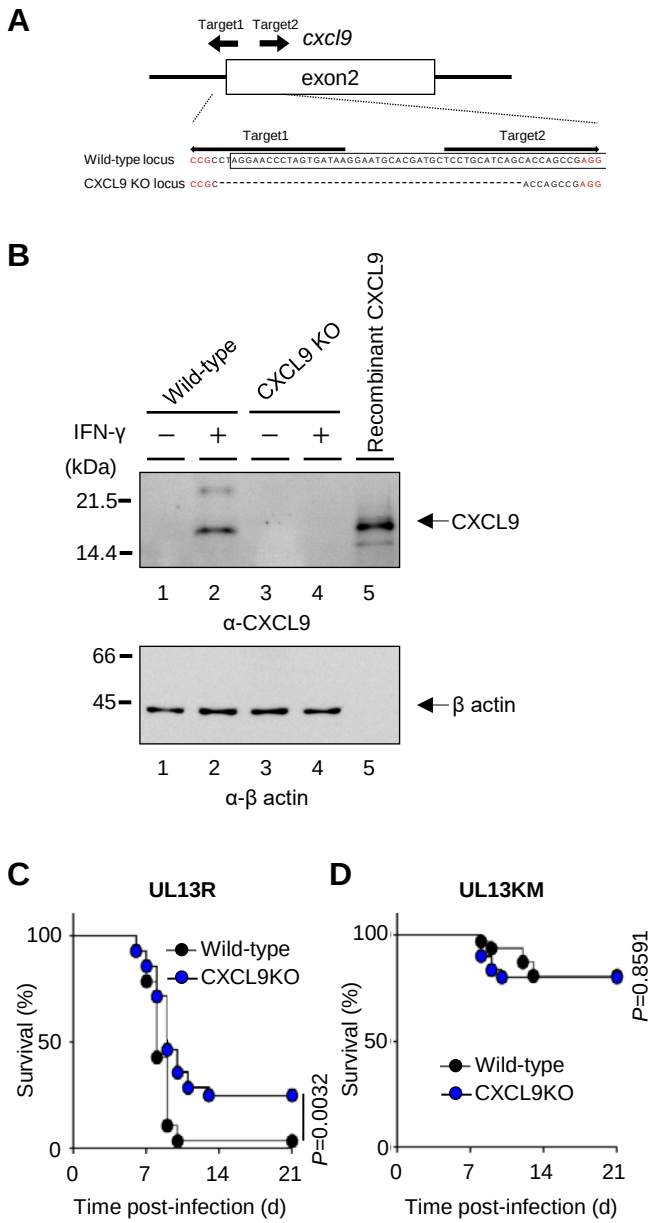
Supplementary Figure 2. Effect of depletion of CD4⁺ T cells on pathogenicity of HSV-1 with and without UL13 kinase activity of mice following ocular infection. (A and B) Five-week-old female ICR mice mock-depleted or CD4⁺ T cell-depleted were infected with 1×10^6 PFU UL13R (A) or UL13KM (B) per eye and monitored for survival daily for 21 d. The results from 2 independent experiments (each with 6 mice) were combined. The statistical significance values were analyzed by the Log-rank test.



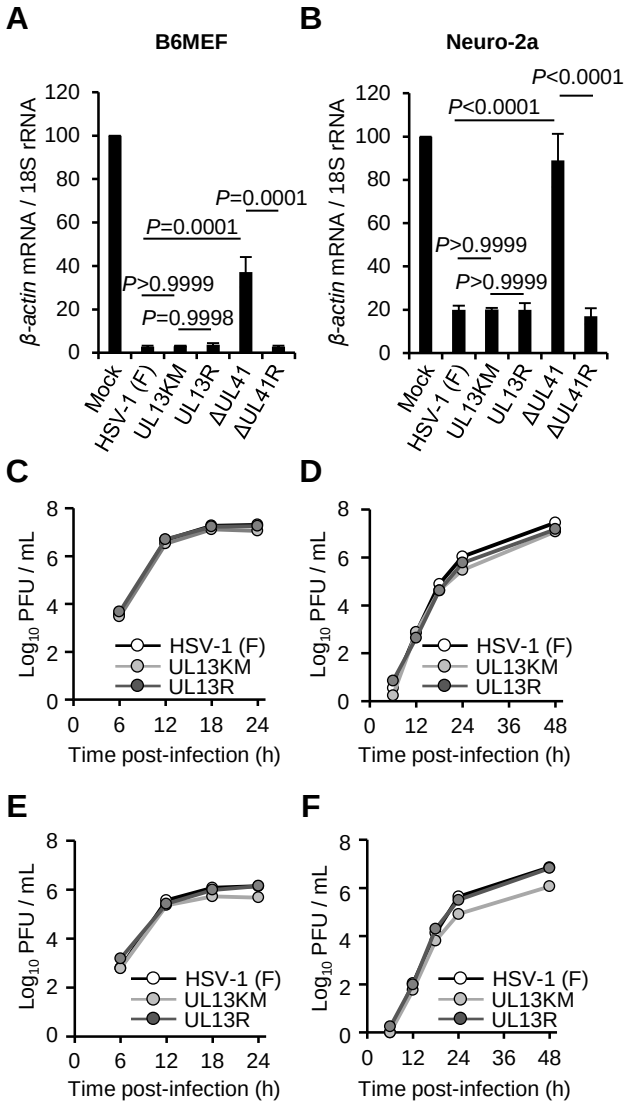
Supplementary Figure 3. Effect of UL13 kinase activity on HSV-1-specific antigen presentation. B6MEFs were mock-infected or infected with wild-type HSV-1(F), UL13KM, UL13R, ΔUs3 or ΔUs3R at an MOI of 1 for 12 h and then co-cultured for an additional 12 h with *lacZ*-inducible CTL hybridoma cells recognizing HSV-1 gB (HSV-2.3.2E2), followed by β-galactosidase assays. Each data point is the mean ± standard error from three triplicate experiments. Statistical significance values were determined by one-way analysis of variance (ANOVA) followed by the Tukey post-test.



Supplementary Figure 4. Effect of direct injection of CXCL10 into the brain stems of mice ocularly infected with UL13R on CD8⁺ T cell accumulation and viral pathogenicity. Five-week-old female ICR mice were ocularly infected with 1×10^6 PFU UL13R per eye. (A to F) At 5 d post-infection, CXCL10 or PBS was injected into the brain stems of the infected mice. At 7 d post-infection, brain stem (A and E) and submandibular lymph node (B and F) samples were processed and analyzed for CD8⁺ T (CD8⁺, CD3⁺ and CD45⁺) (A and B) or CD4⁺ T (CD4⁺, CD3⁺ and CD45⁺) (E and F) cell content by flow cytometry. At 7 d post-infection, CD8⁺ T cells purified from brain stem (C) and submandibular lymph node (D) samples were assayed for IFN-g producing cell content by ELISPOT assays. The results from four independent experiments (each with 4 mice) were combined. Each data point is the number of each type of cells in each tissue of one mouse (A to F). (G) At 5 and 7 d post-infection, viral titers in the brains of infected mice were assayed. The results from three independent experiments (each with 5 mice) were combined. Each data point is the virus titer in the brain of one mouse. (H) Survival was monitored daily for 21 d. The results from four independent experiments (each with 6 mice) were combined. The statistical significance values were analyzed by the Mann-Whitney *U* test (A to G) or the Log-rank test (H).



Supplementary Figure 5. Generation of *Cxcl9* knockout (KO) mice and the effect of CXCL9 knockout on the pathogenicity of HSV-1 with and without UL13 kinase activity of mice following ocular infection. (A) Schematic illustration of the *Cxcl9* gene. Top: Structure around the target loci. Black arrows indicate the target loci of the gRNAs. White box indicates exon 2 of the *Cxcl9* gene. Middle: wild-type sequence of the *Cxcl9* gene around the target loci. The target sequences for each of the gRNAs are indicated by black arrows, and the PAM sequence is labeled in red. Bottom: Knockout sequences of the *Cxcl9* gene around the target loci. The hyphens indicate deletion nucleotides. (B) Primary MEFs from wild-type or *Cxcl9*-knockout mice were mock-treated or treated with 20 ng/ml recombinant mouse IFN-γ. Cells were harvested at 18 h post-incubation and analyzed by immunoblotting with antibodies to CXCL9 and β-actin. Recombinant mouse CXCL9 was used as a control. Data are representative of three independent experiments. (C and D) Five-week-old female wild-type or *Cxcl9*-knockout ICR mice were infected with 1×10^6 PFU UL13R (C) or UL13KM (D) per eye and monitored for survival daily for 21 d. The results from six independent experiments ((C) 1 with 7 mice, 2 with 5 mice, 2 with 4 mice and 1 with 3 mice, or (D) 1 with 7 mice, 1 with 6 mice, 2 with 5 mice and 2 with 4 mice) were combined. The statistical significance values were analyzed by the Log-rank test.



Supplementary Figure 6. Characterization of recombinant viruses UL13KM and UL13R. (A and B) To measure the viral endoribonuclease activity responsible for host protein synthesis shutoff (vhs), B6MEF (A) and Neuro-2a (B) cells were mock-infected or infected with each of the indicated wild-type and recombinant viruses at an MOI of 5, harvested at 24 h post-infection and the amount of *β-actin* mRNA was analyzed by quantitative RT-PCR. Each bar is the mean ± standard error of data from three independent experiments. Statistical significance values were determined by one-way analysis of variance (ANOVA) followed by the Tukey post-test. The mean value for each of the indicated viruses was calculated relative to that for the corresponding mock-infected cells, which was normalized to 100. (C-F) Vero (C and D) and rabbit skin (E and F) cells were infected at an MOI of 5 (C and E) or 0.01 (D and F) with wild-type HSV-1(F), UL13KM or UL13R. Total virus from the cell culture supernatants and infected cells was harvested at the indicated times post-infection and assayed on Vero cells. Data are representative of three independent experiments.